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Determination of the X-ray Crystal Structure of Phosphotransacetylase from *Methanosarcina thermophila*.

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Beamline(s): X9A

Introduction: Phosphotransacetylase is a key enzyme in the pathway of methanogenesis from acetate. In the archaeon *Methanosarcina thermophila*, phosphotransacetylase, along with acetate kinase, activates acetate to acetyl-CoA for eventual cleavage to methane and carbon dioxide [1]. Phosphotransacetylase and acetate kinase also form an important component of the energy-yielding pathway of fermentative bacteria.

Methods and Materials: A native crystal of phosphotransacetylase was soaked in a solution containing 0.1 M sodium acetate, pH 5.0, 2.2 M ammonium sulfate and 0.1 mM tantalum bromide for 20 hours. The crystal was cooled to 100 K and x-ray diffraction data were collected using 1-degree oscillation and an exposure time of 30 seconds per frame. X-ray diffraction was performed at 9882 eV, 9885 eV and 10200 eV.

Results: The phosphotransacetylase crystal used diffracted x-rays to a maximum resolution of 3.5 angstroms. The tantalum bromide derivative used here had the same cell dimensions (114*114*128 angstroms) as the native crystals and belonged to the same space group (I41), indicating that it was isomorphous with the native crystals. The data showed anomalous diffraction at the tantalum edge. The heavy atom positions in the asymmetric unit were found using the direct methods program SnB [2]

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References:

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[2] H.A. Hauptman, "Shake-and-bake: an algorithm for automatic solution *ab initio* of crystal structures," Methods Enzymol., **277**, 3-13, 1997.